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Behavioural evaluation of mouse models of type 2 diabetes

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ABSTRACT

Motivation for food reinforcers in mouse models of type 2 diabetes was examined in three experiments. In all experiments, physiological measures indicated the presence of type 2 diabetes. In Experiment 1, high-fat fed Swiss TO mice and lean controls showed a preference for high-fat corn oil reinforcers over high-sugar syrup reinforcers in a T-Maze task, but there were no differences between the two groups. In Experiment 2, high-fat fed Swiss TO mice and lean controls responded for corn oil or food pellet reinforcers on progressive ratio schedules. While break point numbers were higher for corn oil than food pellets, and satiation or extinction reduced break points, there were no differences between the groups. In Experiment 3, streptozotocin treatment was used to induce type 2 diabetes in C57Bl/6 j mice that were compared with controls while responding for corn oil or food pellet reinforcers on progressive ratio schedules. While break point numbers were higher for corn oil than food pellets before and after streptozotocin treatment, and satiation or extinction reduced break points, there were no differences between the groups. Parameters for a more complex method of assessment of progressive ratio behaviour derived from Killeen's Mathematical Principles of Reinforcement were also computed. While the parameter associated with incentive value was higher for corn oil than for food pellets, parameters were not significantly affected by streptozotocin treatment. Overall, a range of behavioural measures of food motivation failed to reveal effects of changes relative to controls in mice that showed physiological evidence of type 2 diabetes.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder that manifests in hyperglycaemia, insulin resistance and pancreatic beta-cell dysfunction. Estimates from the International Diabetes Federation suggest that around 422 million people worldwide have T2DM, and this number is predicted to rise to around 629 million people by 2045 (Cho et al., 2018). Furthermore, T2DM is associated with several complications including kidney failure, blindness, heart attacks and stroke. As such, T2DM is a major socioeconomic burden and more improved treatment modalities are urgently required.

An underexplored area in diabetes research relates to mechanisms underlying key behaviours specifically in relation to food preference (Wing & Hill, 2001). In this regard, the use of animal models is beneficial to elucidate underlying mechanisms. One popular animal model in diabetes research is the diet-induced model which involves feeding a high-fat diet to rodents over an extended period. This results in progressive weight gain and elevated blood glucose concentrations and replicates the gradual development of obesity induced T2DM evident in humans (Winzell & Åhrén, 2004). The high-fat diet mouse model is widely accepted as a useful model of

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human obesity and T2DM, and is used in the development and evaluation of novel pharmacological treatments in mouse strains including Swiss TO (Gault, McClean, Cassidy, Irwin, & Flatt, 2007; Porter, Kerr, Flatt, Holscher, & Gault, 2010; Porter, Irwin, Flatt, Holscher, & Gault, 2011) and C57Bl/6 j (Saravanan & Pari, 2015). Yet, to date, there have been few attempts to evaluate the specific food preferences of these mice, or any other assessment of behavioural processes involved in food intake, such as motivation to eat. Given that high-fat fed mice become obese and shows signs indicative of T2DM, their motivation to eat has presumably increased. This could occur in various ways but, as will be evident from the literature reviewed below, it is not yet clear what the mechanisms is. It could be the case that high-fat foods are more attractive to T2DM mice because of insulin insensitivity (Levy et al., 2014), or it may be that high carbohydrate foods are more valued because of cellular glucoprivation (Thorens, 2008). As neither of these hypotheses could be rejected based on existing knowledge, this research is exploratory and investigated both possibilities.

An earlier study with Swiss TO mice exposed to a high-fat diet assessed preference between food pellets with 65 % sugar content and 100 % sucrose pellets. The validated procedure used (Leslie, Norwood, Kennedy, Begley, & Shaw, 2012), showed no clear preference for one food over the other (Hitchen, 2012). It may be that the method is not sufficiently sensitive, or that preference for high-calorie sweetened food does not increase, or that preference shifts to high-fat foods. The latter would be consistent with findings from some human food preference studies (e.g., Drewnowski, Brunzell, Sande, Iverius, & Greenwood, 1985; Elfhag & Erlanson-Albertsson, 2006), and is indirectly supported by a palatability test. Yoneda et al. (2007) found that mice maintained on a standard laboratory chow diet showed higher preference for stronger concentrations of corn oil (highest preference was for 100 % corn oil). Therefore, it may be useful to directly compare choice of a high-fat food or a high-sugar food in a high-fat diet mouse model. This was attempted in Experiment 1, employing Swiss TO mice exposed to a high-fat diet to mimic mild-moderate hyperglycaemia, where a T-maze was used as it provides a simple measurement of choice or preference between two simultaneously available foods. In recent years, the apparatus has primarily been used to assess memory (e.g., Patil, Sunyer, Höger, & Lubec, 2009). However, in its original conception the T-maze was used to evaluate preferences between small quantities of varying food types in order to provide an indication of taste preference, without the need to consider post-ingestive effects of larger quantities of foods (Young, 1936). Measurements of preference include more frequent selection of food type, time taken to run to food choice and time taken to consume food (Stellar, 2012).

Experiments 2 and 3 used operant conditioning, which provides powerful methods for the measurement of motivation (Hånell & Marklund, 2014). Fixed ratio (FR) schedules have been used extensively to assess the rewarding effects of drugs and food (e.g., Pickens & Thompson, 1968), but may have limited utility for measuring motivation. Arnold and Roberts (1997) noted that behaviour maintained by FR schedules was insensitive to effects of reinforcer magnitude (see also Richardson & Roberts, 1996) and suggested that the progressive ratio (PR) schedule is more appropriate for assessing appetitive motivation. In PR schedules, the required number of responses for reinforcement is initially low and increases systematically within a session (Hodos, 1961). During lower ratios, responding on PR schedules is generally well maintained, but as the ratio requirement progressively increases, the rate of responding decreases until the so-called “break point” is reached and responding stops in that session (Bradshaw & Killeen, 2012). PR schedule break point has been used as a measure of the motivational state of the subject (Barr & Phillips, 1999), and of the magnitude and incentive value of the reinforcer (Cheeta, Brooks, & Willner, 1995; Rickard, Zhang, Body, Bradshaw, & Szabadi, 2009). As a linear increasing series of ratios (e.g., 5, 10, 15 etc.) may lead to satiation with food and not maintain a stable rate of responding across sessions (Finger, Dinan, & Cryan, 2010), Richardson and Roberts (1996) used a series where the response requirement increases exponentially over successive ratios. Derived from r (response ratio, rounded to nearest integer) = $[5e^{(\text{step number} \times 0.2)}] - 5$, the exponential PR schedule consists of these response requirements: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603 etc. The exponential PR schedule results in steadier response rates, with higher break points achieved, compared to linear PR schedules (Killeen, Posadas-Sanchez, Johansen, & Thrailkill, 2009), and exponential PR schedules maintain stable response rates in mice that fail to reach response stability under a linear PR schedule (Finger et al., 2010).

In a relevant human experimental study, Miras et al. (2012) tested obese patients before and after gastric by-pass surgery and found that the break point of responding for high sugar and fat candies on a geometric PR schedule was significantly reduced by gastric by-pass surgery. With rodents, Goto-Kakizaki rats with T2DM had lower break points than non-diabetic controls (Moreira, Malec, Östenson, Efendic, & Liljequist, 2007) and a study with an obese mouse model found that obese mice and lean controls showed equal motivation as both groups reached similar break points (Finger et al., 2010). However, neither of these studies looked at the effects of diet or reinforcer quality. In an unpublished study, Hitchen (2012) assessed the motivation to respond for a sweetened reinforcer in high-fat fed mice and found that these were less motivated to respond on an exponential PR schedule than mice fed on a standard diet. Experiment 2 replicated the procedure used by Hitchen (2012). High-fat and standard diet fed mice from Experiment 1 were trained on an exponential PR schedule for food reinforcers and then for corn-oil reinforcers. In both phases, satiation challenges were implemented, and each phase terminated with several extinction sessions. Across the experiment, there were several occasions where the motivational differences between high-fat fed and standard diet mice could be assessed.

To extend the results of Experiment 2, Experiment 3 used a more detailed analysis of PR performance, and an alternative mouse model of T2DM. Bradshaw and Killeen (2012) devised a quantitative method for analysing motivation of operant behaviour which distinguishes between effects of reinforcers on motivational and motor processes (Valencia-Torres, Bradshaw, Bouzas, Hong, & Orduña, 2014), and is based on Killeen (1994) Mathematical Principles of Reinforcement account of schedule-controlled behaviour. This has three principles: 1) behaviour is activated by reinforcers; 2) the rate of operant responses emitted by the organism is biologically constrained by its physical capacities; and 3) specific reinforcement schedules vary in their effectiveness in coupling reinforcers to operant responses. Ratio schedules of reinforcement generate a distinctive pattern of behaviour wherein a post-reinforcement pause (PRP) with no responses is followed by a period of high rate responding (Leslie, 1996). Bradshaw and Killeen's method requires that PRP and the subsequent running response rate (i.e., the number of responses in that ratio/time taken to

complete them) be collected for each ratio in the PR sequence, along with the overall response rate for each ratio. From these data, Bradshaw and Killeen (2012) derived four parameters whose values are used to provide separate indications of the organism's motor functioning ability and of the reinforcer's motivational impact (Valencia-Torres et al., 2014). These parameters are: alpha (α), a motivational parameter called specific activation; delta (δ), a response time parameter which defines the minimum amount of time that must pass between two successive responses; T_0 , the minimum pause after any reinforcer; and k , a parameter that reflects the sensitivity of the PRP to the progressively increasing ratio demand of the schedule.

The alternative model of T2DM used in Experiment 3 was the streptozotocin (STZ)-induced diabetic rodent, which typically mimics more severe hyperglycaemia, wherein abnormally elevated blood glucose levels are provoked through administration of STZ. The severity of diabetes may be altered depending on the dose (Junod et al., 1967), and although it is less damaging than other models of chemically induced diabetes (Srinivasan & Ramarao, 2007) there are risks of mortality. To balance the efficacy of inducing abnormally high blood glucose levels with lower mortality rates, Gault et al. developed an alternative model of STZ-induced diabetes (Gault et al., 2007; Millar et al., 2016, 2017; Porter et al., 2011). This involves initial administration of a single moderate-to-low dose of STZ (50 mg/kg) followed by additional low doses where required until all mice reach the criterion to confirm the presence of diabetes. STZ-treated rodents become hyperglycaemic and hypoinsulinemia and have reduced body weights (Sevak, Koek, Galli, & France, 2007; Srinivasan & Ramarao, 2007). Behaviourally, this animal model has been associated with learning and memory deficits, as STZ-treated mice display difficulty in the acquisition of complex tasks (e.g., shuttle box avoidance) and decreased memory retention of a previously learned task that may be reversed by treatment with insulin (Flood, Mooradian, & Morley, 1990). STZ-induced diabetes has also been shown to affect eating habits. STZ treatment in rats led to a marked increase for food and fluid intake, while simultaneously lowering preference for high concentrations of sugar (Smith & Gannon, 1991). This suggests that although STZ-induced diabetes increases general food consumption levels, this greater appetite is correlated with a lower motivation to obtain food. STZ-induced diabetes also reduces the behavioural effects of dopaminergic drugs (Sevak et al., 2007), and decreases motivation for wheel-running (Howarth, Huang, Roberts, Lin, & McCrory, 2007). Using the STZ-induced mouse model of T2DM, Experiment 3 looked at behaviour on exponential PR schedules maintained by food pellets or corn oil, and its disruption by satiation challenges and extinction.

2. Materials and methods

2.1. Experiment 1

2.1.1. Animals

Fourteen adult Swiss TO mice (Harlan, Blackthorn, UK) were individually housed in temperature-controlled conditions ($22 \pm 2^\circ\text{C}$; 12-h light/dark cycle; lights on from 8:00 am) with *ad libitum* access to food and water. At least 18 weeks prior to the beginning of experimental testing, one group ($n = 8$) were placed on a high-fat diet (45 % fat, 20 % protein, and 35 % carbohydrate; Special Diets Service, Essex, UK), while the other group ($n = 6$) had free access to standard rodent maintenance diet (10 % fat, 30 % protein, and 60 % carbohydrate; Trouw Nutrition, Cheshire, UK). Prior to behavioural experimentation, blood glucose was measured using an automated hand-held Ascensia Contour Blood Glucose Monitoring System (Bayer Healthcare, UK). All animal care procedures were conducted in accordance with the UK Animals Scientific Procedures Act 1986 and its associated guidelines. Prior to Experiment 1, mice on high-fat diet exhibited significantly higher glucose concentrations (11.75 ± 3.12 mmol/l vs 4.90 ± 0.98 mmol/l; $p < 0.001$) compared to mice on standard diet measured in the non-fasting state. Mice on high-fat diet also had higher body weights ($M = 58.0$ g, $SD = 2.83$) than the standard-diet fed mice ($M = 53.1$ g, $SD = 1.90$; $t(11) = 3.59$, $p < 0.01$, independent-samples *t*-test).

2.1.2. Apparatus

Following Deacon and Rawlins (2006), a T-maze was constructed out of dark grey aluminium and consisted of a floor, a starting arm and two goal arms. The starting arm measured 55×10 cm, while each of the two goal arms measured 30×10 cm. All three arms were enclosed by 15 cm high walls. A sliding guillotine door was placed at the entrance to each of the goal arms. At the far end of each goal arm was an aluminium food well (1×1 cm), placed 3 cm in from the end wall. The T-maze was placed on a wooden table, elevated 90 cm from ground level. A white noise generator was used to mask external noise. The two food types used were Golden syrup (Lyle's, UK) and Corn oil (Mazola® pure corn oil, Princes Ltd, UK). The quantity of food used in each trial (during both training and testing) had equal calorific value (0.40 ± 0.02 kcal); 0.05 mL Corn oil and 0.12 mL Golden Syrup.

2.1.3. Procedure

2.1.3.1. Habituation. To habituate mice to the taste of each food type, 1.5 mL of each was placed in small weigh boats inside the home cage for at least 24 h. After this, each mouse was placed in the T-maze for four 3-min periods with at least 10 min between each exposure.

2.1.3.2. Forced-choice training. Prior to training, corn oil was randomly allocated to the left goal arm while golden syrup was allocated to the right goal arm. Each mouse was placed in the start area facing away from the choice point, with selected goal arm door raised and allowed to enter the arm and allotted 5 min to consume food. On the next trial, the procedure was repeated with the other arm. Mice received two trials per day over three consecutive days.

2.1.3.3. Free-choice testing. At the start of each free-choice trial, the mouse was placed in the start area facing away from the choice point. Both goal arm doors were raised. Once the mouse had entered an arm, the door to that arm was then closed. The mouse was removed when the food had been consumed or 3 min had elapsed. All animals received 2 free-choice trials per day (3 on the first day), with a minimum inter-trial interval of 2 h, over 12 consecutive days, totalling 25 free-choice trials. Arm choice and time taken to consume food were recorded.

2.2. Experiment 2

2.2.1. Animals

Twelve mice from Experiment 1 (high-fat fed, $n = 7$ and standard chow diet fed, $n = 5$) were maintained on *ad libitum* water and food until fourteen days prior to training. Blood glucose concentrations, measured in the non-fasting state, were significantly higher in high-fat compared to standard chow mice (12.24 ± 2.70 mmol/l vs 5.06 ± 0.75 mmol/l; $p < 0.001$). From this point, mice were weighed daily and each group maintained at between 80 % and 90 % of their free-feeding weight by providing 3–4 g (depending on individual mouse weights) of high-fat diet and 4–4.5 g of standard chow. During acquisition, FR, PR training, and satiation phases, sessions were conducted five days a week with *ad libitum* access to food being made available at the weekends to the standard diet group, while the high-fat diet group received approximately 90 % of their typical free-feeding intake. Sessions were conducted in this manner to prevent illness from nonspecific causes. During extinction phases, sessions were run daily.

2.2.2. Apparatus

Mouse operant chambers (Med. Associates model No. ENV /307A, Med Associates Inc, St. Albans, VT, USA) were enclosed in sound-attenuating boxes with electric fans. Only the left lever was in use throughout all phases of the experiment, while the right lever was permanently retracted. Initially, reinforcers were 20-mg Noyes food pellets (13 % fat, 20 % protein, and 67 % carbohydrates; Research Diets Inc, New Brunswick, NJ, USA), delivered to a recessed tray located at the bottom of the instrument panel. In a later stage (see Procedure), chambers were fitted with peristaltic pumps (Ismatec model No. MS-CA 2/620, Cole-Parmer GmbH, Futtererstr, Wertheim, Germany) to deliver corn oil (100 % fat, 0 % protein, 0 % carbohydrates; Mazola Pure Corn Oil, Princes Ltd, Liverpool, UK) to a recessed tray situated at the bottom of the instrument panel, via silicone tubing (Tygon SI, Ismatec part No. SC0600, IDEX Health & Science GmbH, Futtererstr, Wertheim, Germany). The volume for each reinforced lever press was 9 microlitres, approximately equal in calorific value to one Noyes pellet. During a satiation procedure, golden syrup (0 % fat, 1 % protein, 78 % carbohydrates; Lyles Golden Syrup, T&L Sugars Ltd, London, UK) was also used along with corn oil, and either food type was presented in 86 mm \times 86 mm \times 25 mm disposable polystyrene weighing dishes (Sigma-Aldrich, St. Louis, MO, USA) and placed at one end of each animal's home cage. A computer programmed in Med.-PC controlled the procedure and recorded presses on the retractable lever.

2.2.3. Procedure

2.2.3.1. Free-operant acquisition with food pellet reinforcement. During five 30-min sessions, the house light was on and the retractable lever was permanently extended. A food pellet was delivered immediately following a lever press (FR1), and on average every minute independent of behaviour. A buzzer sounding accompanied the delivery of each pellet.

2.2.3.2. FR1 training. A lever press resulted in one pellet delivery while simultaneously causing the buzzer to sound and the lever to retract. For these and all subsequent sessions, the interval prior to lever re-insertion was 30 s, and the house lights were permanently on. A session terminated once 20 reinforcers were obtained by lever presses, or if 30 min elapsed. Mice that obtained 20 reinforcers on two consecutive days following at least six days moved on to PR training.

2.2.3.3. PR training. Completion of the PR requirement resulted in lever retraction and a buzzer to sound. Mice were required to complete response requirement, r , according to $r = (5 \times e^{0.2n}) - 5$, rounded to the nearest integer, with n representing the position in the sequence of ratios (Roberts & Richardson, 1992). This resulted in PR values as follows: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737 (the highest ratio reached). The session terminated if the current ratio was not completed in 15 min. The last ratio to be completed before session termination was defined as the break point. Each animal received a minimum of eight sessions and responding was deemed as stable when ratios completed on each session was within 10 % of the mean of the last three sessions.

2.2.3.4. Satiation probe 1 and retraining. Then followed 48-h *ad libitum* access to food with any remaining food being removed two hours prior to one PR session. The mouse was then returned to the previous original restricted diet before further PR sessions until stability was attained. This occurred within four sessions for all mice, after which there was a series of extinction sessions.

2.2.3.5. Extinction. The same PR schedule was in effect. Completion of a ratio resulted in the lever retracting and the buzzer sounding, but now no pellet was delivered. Session ended when 15 min had elapsed without completion of a ratio. Extinction sessions lasted from 17 to 113 min, for 11 daily sessions. There followed a 3-week break. Both groups were allowed *ad libitum* access to their respective diets for seven days. Thereafter, mice were placed on the same restricted diet as before and were maintained at between 80 % and 90 % of their free-feeding weight by providing 3–4 g of high-fat diet for the high-fat group and 4–4.5 g of standard rodent maintenance chow

to the standard diet group once daily.

2.2.3.6. PR retraining with corn-oil reinforcement. Sessions replicated the same procedure of the earlier PR training, but with corn-oil reinforcement. Training continued until the same stability criterion was met.

2.2.3.7. Satiation probe 2 and retraining. This was as for Satiation Probe 1 and retraining.

2.2.3.8. Satiation Probes 3 and 4 and retraining. Mice were preloaded with different amounts of either oil or syrup prior to a PR session. The amount of either food type used was 1.2 kcal, approximately equal calorific content to 20 Noyes pellets (the maximum number available during FR1 training sessions) and comprised 0.13 mL corn oil or 0.51 mL of golden syrup. For Satiation Probe 3, mice from each group were randomly allocated to the corn oil preloading condition (high-fat, $n = 4$; standard, $n = 2$), while the remaining mice were allocated to the golden syrup condition (high-fat, $n = 3$; standard, $n = 3$). Either food type was placed in the animal's home cage 1 h prior to testing on PR schedule. There followed two sessions of PR training without preloading. For Satiation Probe 4, preloading conditions were reversed. There followed two sessions of PR training without preloading.

2.2.3.9. Satiation probe 5 and retraining. During Satiation Probes 3 and 4, some mice from either dietary group failed to consume all the syrup provided. Consequently, there was a final probe with only corn oil used. The volume was greatly increased and approximately equal to half of the average daily free-feeding calorific intake for each dietary group. Data from our lab showed that high-fat diet Swiss-TO mice consume an average of 43.78 kcal per day, whereas standard-diet fed Swiss-TO mice consume an average of 23.71 kcal per day. Thus, high-fat mice received were given 2.3 mL (21.7 kcal) and standard-diet mice given 1.25 mL (11.8 kcal) of corn oil in weighing dishes placed in the home cage 1 h prior to a PR session. Mice were then retrained on the PR schedule until stability in responding was achieved, which occurred within five or six sessions.

2.2.3.10. Extinction. There followed 5 extinction sessions, using the same procedure as in the previous extinction phase. Extinction sessions lasted from 17 to 63 min.

2.3. Experiment 3

2.3.1. Animals

Thirty-six male C57Bl/6 j mice (23–30 g; >12 weeks old; Harlan UK Ltd., Blackthorn, UK) were housed under the same conditions as outlined above and all received normal chow. Four weeks prior to experimental studies, mice were maintained between 80 % and 90 % of their free-feeding weight by providing 3–3.5 g of standard maintenance chow once daily. After the last pre-treatment PR training session (see below), mice were fasted for 24 h before receiving streptozotocin (STZ) or vehicle. STZ (Sigma-Aldrich, Dorset, UK) diluted in 0.1 M sodium citrate buffer (pH 4.5) was injected (50 mg/kg bw; ip) to approximately half of the mice ($n = 17$) with remaining mice ($n = 19$) receiving saline vehicle (0.9 % wt/vol). Immediately before injections were given, baseline measurements of blood glucose concentrations were recorded and then one week later. Mice in the STZ treatment group with blood glucose <10 mmol/l received an additional injection of STZ (30 mg/kg). Water consumption was recorded during the post-treatment progressive ratio training and extinction phases. Blood glucose levels, measured in the non-fasting state, were significantly higher for STZ-treated mice (15.46 ± 6.74 mmol/l) compared to vehicle controls (6.01 ± 0.68 mmol/l), $t(37) = 6.07$, $p < 0.001$ by an independent-samples t -test. Body weights were taken immediately prior to first administration of injections and one week after the end of the study (five weeks later). Body weights prior to first administration were very similar for the two groups (STZ group $M = 28.4$ g, $SD = 1.92$, control group $M = 28.2$ g, $SD = 1.88$) but different one week after the end of the study (STZ group $M = 26.8$ g, $SD = 1.66$, control group $M = 28.6$ g, $SD = 1.84$). Repeated measures ANOVA was carried out with one within-subjects factor (time) and one between-subjects factor (treatment). There was no effect of treatment. However, there was an effect of time ($F_{1,34} = 7.51$, $p < 0.05$), indicating that weights changed across times and an interaction between time and treatment ($F_{1,34} = 16.44$, $p < 0.001$), showing that weights of STZ-treated mice decreased across times. Water intake was measured over the last 11 days of experiment and independent-samples t -test on mean water intakes (mL/day) demonstrated a significant difference, where STZ-treated mice consumed more water (9.64 ± 6.41 mL) compared to vehicles (6.38 ± 1.12 mL); $t(34) = 2.19$, $p < 0.05$. During PR training phases, sessions were only conducted five days a week with mice receiving ad libitum access to food at the weekends to prevent illness from nonspecific causes.

2.3.2. Apparatus

Mouse operant chambers from the previous study were used. Twenty chambers were set to deliver standard 20 mg Noyes pellets for half the mice, and two chambers were calibrated to deliver 9 μ L (with the same calorific value) of corn oil for the other mice. A computer programmed in Med-PC controlled the procedure, reinforcer delivery, and the recording of lever presses on the retractable lever. Digital laboratory scales and automated hand-held blood glucose monitoring system were used to measure mouse body weights and blood glucose levels, respectively.

2.3.3. Procedure

2.3.3.1. Free-operant acquisition. as in Experiment 2, five daily free-operant acquisition sessions ran for 30 min. The house light was

on, with the retractable lever permanently extended, for the duration of each session. Mice were randomly allocated to one of two groups, with half of mice ($n = 18$) responding for a food pellet reinforcer with the other half ($n = 18$) responding for a corn oil reinforcer. A food pellet or small quantity of corn oil ($9 \mu\text{L}$) was delivered following each lever press, and on average every minute independent of behaviour. The delivery of either reinforcer was accompanied by a buzzer sounding.

2.3.3.2. FR 1 training. This was as in Experiment 2.

2.3.3.3. PR training. This was as in Experiment 2, except that the interval between ratios was reduced to 10 s and sessions terminated after 60 min or if the current ratio was not completed in 15 min. Mice took a maximum of 10 sessions to reach stability criterion.

2.3.3.4. STZ treatment and PR training. Mice from each group were allocated to a STZ or vehicle treatment group, depending on mouse body weight and performance during prior PR training. Approximately half of the mice from each group received STZ injections. Mice then returned to PR training (procedure as above) and took a maximum of 10 sessions to reach stability criterion.

2.3.3.5. Extinction. The procedure was as in Experiment 2, except that the interval between ratios remained at 10 s. There were four extinction sessions.

2.3.4. Additional behavioural measures

The overall response rate was calculated for each ratio by dividing the number of responses by the total time taken to complete the ratio, including the post-reinforcement pause. Total time was measured from the end of the preceding reinforcer delivery until the emission of the last response of the ratio (Bizo & Killeen, 1997). Data from the first ratio (one response) and any uncompleted ratios at the end of the session were not subject to analysis. Running rate was calculated by dividing the number of responses by the 'run time', which was defined as the time taken to complete the ratio, excluding the post-reinforcement pause. In each case, the post-reinforcement pause was measured as the time taken for mice to respond following re-insertion of the lever after the ITI from completing the previous ratio. Estimates of the four parameters (T_0 , k , a , and δ) were then derived using the 'Solver' facility of Microsoft Excel using a program supplied by P. Killeen. The resulting data from each of the conventional measures and each of the four parameters were then submitted to a general statistical programme (SPSS), where an analysis of variance (ANOVA) with repeated measures was carried out. The Greenhouse-Geisser correction was used for analysis where the criterion for homogeneity was not met.

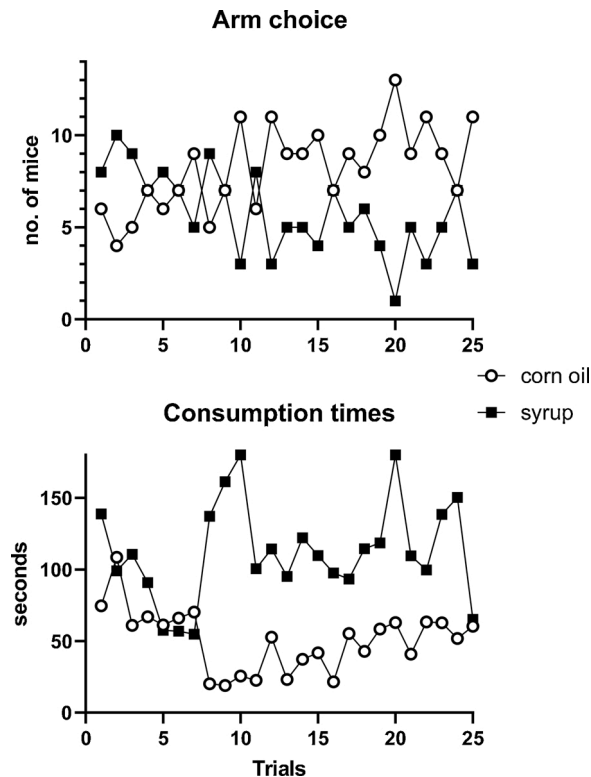


Fig. 1. Upper panel: Number of mice selecting the corn-oil arm (red symbols) or syrup arm (green symbols) over 25 trials of T-maze experiment. Lower panel: Average times taken for mice to enter a T-maze arm and consume either food type over 25 trials.

3. Results

3.1. Experiment 1

Over the last 10 trials both groups showed a preference for the corn oil T-maze arm, selected by the high-fat group ($n = 8$) on 72.5 % of trials and by the standard diet group ($n = 6$) on 63.6 % of trials. Choices were compared on a trial by trial basis for the final 10 trials and there was no difference between the groups (by a Mann-Whitney test, $n_1 = n_2 = 10$, $U = 32$, $p > 0.05$). As there was no effect of diet, other analyses were performed for the two groups taken together. Fig. 1 (upper panel) shows the number of mice choosing each arm across all 25 trials. While in early trials there was no clear preference, over the last 10 trials preference had shifted to the corn-oil arm (by a Mann-Whitney test, $n_1 = n_2 = 10$, $U = 2$, $p < 0.01$). Fig. 1 (lower panel) shows mean consumption times for all trials for corn-oil and golden syrup when selected. There was an upward trend in syrup consumption times and a downward trend in corn-oil consumption times, and over the last 10 trials syrup times were longer than corn-oil times (by a Mann-Whitney test, $n_1 = n_2 = 10$, $U = 0$, $p < 0.01$).

3.2. Experiment 2

3.2.1. Food-pellet PR training

Body weights at the beginning of training were higher for the high-fat fed mice diet ($M = 57.9$ g, $SD = 2.67$) than for the standard-diet fed mice ($M = 49.1$ g, $SD = 2.93$); $t(11) = 5.66$, $p < 0.001$ by independent-samples t -test). The group mean break point numbers of reinforcers received for each PR session and effects of diet, satiation probes and extinction are shown in Fig. 2. An ANOVA was carried out for the last five food pellet PR sessions of the initial training phase, with one within-subjects factor (session), and one between-subjects factor (diet). There was no main effect of diet or sessions and no interaction, thus both groups of mice were reaching a similar break point of around 17 reinforcers per session. For the first satiation probe, an ANOVA with one within-subjects factor (session) and one between-subjects factor (diet) was carried out for the prior session of PR training and the two sessions following 48 h feeding. There was no main effect of diet but there was an effect of session ($F_{2,16} = 24.98$, $p < 0.001$). Pairwise comparison between sessions showed that break point numbers in both sessions after satiation were lower than those in the prior session ($p < 0.001$ in each case). An ANOVA with one within-subjects factor (session) and one between-subjects factor (diet) was performed for all 11 extinction sessions. There was a main effect of sessions ($F_{10,100} = 5.67$, $p < 0.001$), but no difference between groups or interactions between factors, showing that behavioural extinction occurred across sessions and did not differ between groups. As evident from Fig. 2, break point number declined from around 15 to around 8 after 11 extinction sessions.

3.2.2. Physiological data prior to corn-oil reinforcement

High-fat diet mice had higher blood glucose concentrations (12.37 ± 2.99 mmol/l) compared to standard diet mice (5.07 ± 0.92 mmol/l), $t(7) = 6.14$, $p < 0.001$ (by independent-samples t -test) and similar to those obtained at the beginning of the experiment. By an independent samples t -test, there was a significant difference in body weights of mice fed a high-fat diet (50.09 ± 2.37 g) to those on standard diet (45.64 ± 1.47 g), $t(10) = 3.68$, $p < 0.01$.

3.2.3. Corn-oil PR training

An ANOVA of the last five PR sessions of initial training with one within-subjects factor (session), and one between-subjects factor

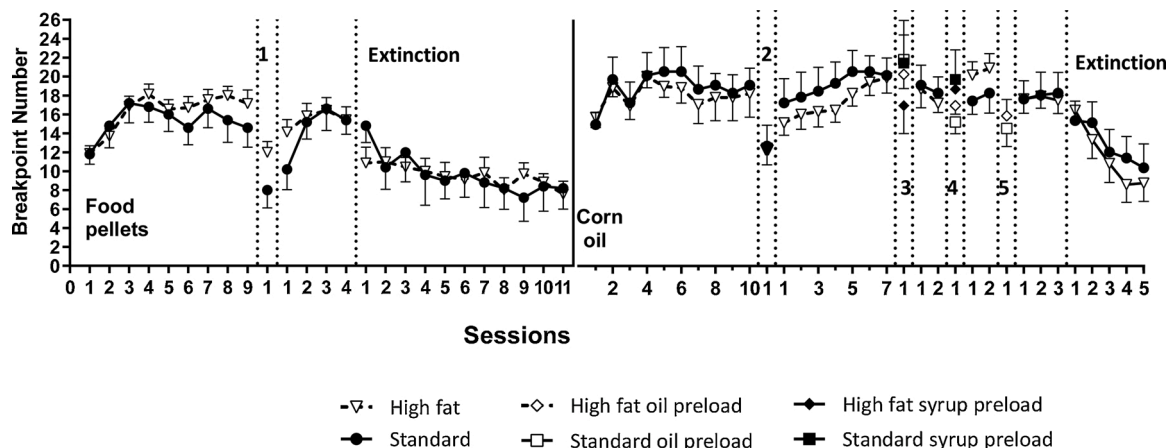


Fig. 2. Left panels: Group mean break point values for Swiss-TO mice fed on a high-fat diet or standard diet responding for food pellets, following satiation (1), retraining and extinction. Right panels: Group mean break point values for Swiss-TO mice fed on a high-fat diet or standard diet responding for corn oil, following satiation (2), retraining interspersed with preloads (at 3, 4, and 5, see text for details) and extinction. Bars indicate standard errors.

(diet), showed no effects of sessions, diet or interaction between these factors. Mean values, at around 19 reinforcers obtained, were higher than for training with food-pellet reinforcement. The effect of Satiation Probe 2 was examined by ANOVA, with one within-subjects factor (session) and one between-subjects factor (diet), for the prior session and two subsequent sessions. There was no effect of diet but there was a main effect of session ($F_{2,13} = 14.30, p < 0.001$). Pairwise comparisons showed that break point numbers in the probe session were lower than those before and after ($p < 0.001$ in each case). The effect of Satiation Probes 3 and 4 were examined by ANOVA, with one within-subjects factor (session) and one between-subjects factor (diet). As animals failed to complete consumption in the home cage when preloaded with syrup, only data from the pre-load with oil condition were analysed. There was no significant effect of diet but there was a main effect of session ($F_{2,20} = 10.45, p < 0.01$) with pairwise comparison between sessions showing that break point numbers in two sessions after pre-load were lower than in the prior session ($p < 0.05$ in each case). An ANOVA with one within-subjects factor (session) and one between-subjects factor (diet) was performed for all five extinction sessions. There was a main effect of sessions ($F_{4,40} = 19.16, p < 0.001$), but no difference between groups or interactions between factors, thus behavioural extinction occurred across sessions and did not differ between groups. As evident from Fig. 2, mean break point number declined from around 15 to around 9 after 5 extinction sessions.

3.3. Experiment 3

3.3.1. PR training

Fig. 3 shows mean break point numbers on the PR schedule for pre-treatment, post-treatment and extinction sessions. An ANOVA for the final five sessions of the pre-treatment phase with one within-subjects factor (session) and one between-subjects factor (reinforcer) showed an effect of reinforcer ($F_{1,34} = 18.43, p < 0.001$), with pairwise comparison between groups indicating that mice responding for corn oil reinforcement reached higher break point numbers than mice responding for pellet reinforcement ($p < 0.001$). There was no interaction between factors. An ANOVA for the final five sessions of the post-treatment phase with one within-subjects factor (session) and two between-subjects factor (reinforcer and treatment) showed main effects of sessions ($F_{3,86} = 7.11, p < 0.001$) and reinforcer ($F_{1,32} = 8.21, p < 0.01$), with pairwise comparison showing higher break point numbers for mice responding for corn oil reinforcement than mice responding for pellet reinforcement ($p < 0.01$). There was no main effect of treatment or interactions between any factors. An ANOVA for the four extinction sessions with one within-subjects factor (session) and two between-subjects factor (reinforcer and treatment) again showed main effects of sessions ($F_{3,96} = 31.55, p < 0.001$) and reinforcer ($F_{1,32} = 13.32, p < 0.005$), with pairwise comparison showing that break point numbers were lower for mice that were previously reinforced with pellets than mice that were previously reinforced with corn oil. There was no effect of treatment or interaction between factors. Overall, this indicated that behavioural extinction occurred across sessions and differed between reinforcer conditions but not between treatment groups.

3.3.2. Parameters of the quantitative analysis

The Bradshaw and Killeen (2012) model provided a moderate fit of the overall and running response rate data from both groups and each condition (pre-treatment: vehicle/standard pellet, $r^2 = 0.64$; vehicle/corn-oil, $r^2 = 0.65$; STZ/standard pellet, $r^2 = 0.60$; STZ/corn-oil, $r^2 = 0.58$; post-treatment: vehicle/standard pellet, $r^2 = 0.38$; vehicle/corn-oil, $r^2 = 0.50$; STZ/standard pellet, $r^2 = 0.53$; STZ/corn-oil, $r^2 = 0.63$). Table 1 shows the mean values of all four parameters from mice responding for either reinforcer type for the final five sessions of the pre-treatment phase and for the final five sessions of the post-treatment phase.

A two-way ANOVA of the pre-treatment values showed that none of the parameters differed significantly between STZ- and vehicle-treated groups. However, there were significant effects of reinforcer type on two of the four parameters, with an increase of the k parameter in the food pellet group ($F_{1,35} = 41.98, p < 0.001$) and a reduction of the α parameter in the food pellet group ($F_{1,35} = 9.68, p < 0.005$). To compare changes of parameter values following treatment, a mixed ANOVA was conducted with one within-subjects

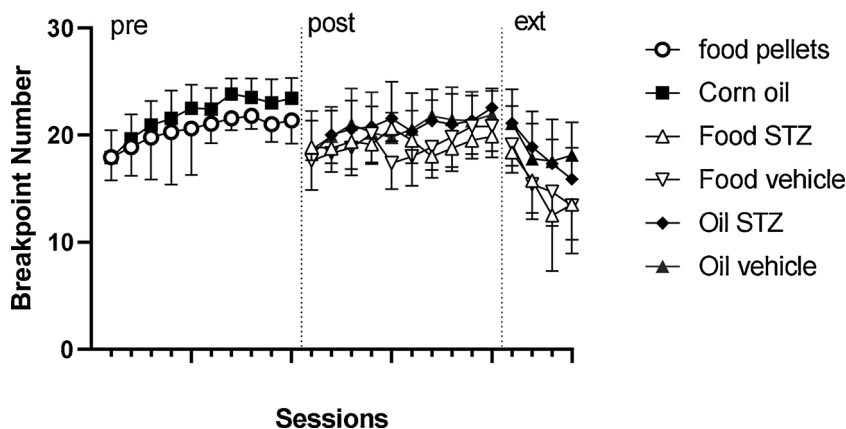


Fig. 3. Group mean break point values for C57Bl/6 j mice responding for food pellets or corn oil before (pre) and after (post) STZ or vehicle treatment, and then during extinction. Bars indicate standard errors.

Table 1

Pre- and post-treatment values of all four parameters of the PR model for vehicle- treated and STZ-treated mice. Standard deviation in parenthesis.

Vehicle-treated groups			STZ-treated groups		
Parameter		Corn oil	Food pellet	Corn oil	Food pellet
T_0	Pre	5.49 (3.25)	11.22 (8.33)	9.55 (6.73)	9.27 (7.37)
	Post	11.73 (12.43)	11.39 (8.55)	9.78 (7.66)	13.45 (5.88)
k	Pre	0.09 (0.09)	0.25 (0.10)	0.07 (0.10)	0.37 (0.13)
	Post	0.25 (0.17)	0.56 (0.20)	0.23 (0.20)	0.56 (0.17)
a	Pre	33.09 (28.00)	13.23 (6.71)	29.40 (14.00)	16.37 (10.95)
	Post	17.33 (9.65)	33.62 (24.39)	18.65 (19.75)	12.77 (6.21)
δ	Pre	4.45 (0.68)	3.81 (1.20)	4.84 (1.42)	3.58 (1.27)
	Post	3.66 (0.67)	4.91 (1.10)	3.59 (1.14)	3.06 (0.82)

factor (phase: pre- and post-treatment) and two between-subject factors (treatment and reinforcer type) for each of the four parameters. Results for the T_0 parameter demonstrated no significant main effect of phase, treatment, or reinforcer type. There was a significant main effect of phase on k ($F_{1,32} = 75.57, p < 0.001$), showing an increase of this parameter in the post-treatment phase ($p < 0.001$) and a main effect of reinforcer type ($F_{1,32} = 37.08, p < 0.001$), with pairwise comparison indicating an increase of k for the food pellet reinforcer ($p < 0.001$). There was no effect of treatment or interaction between any of the factors. For the parameters a and δ , there were no main effect of phase, treatment, or reinforcer type, or interaction. To assess effect of extinction on parameter values, a mixed ANOVA with one within-subjects factor (session) and two between-subjects factors (treatment and reinforcer type) was conducted using the mean of the final five sessions of post-treatment (termed session 1) along with those from the first 3 extinction sessions for all parameters (see Table 2). The final extinction session was excluded from analysis due to unstable data. Results showed no main effect of session, treatment, or reinforcer type for the T_0 parameter. There was a main effect of reinforcer type on k ($F_{1,32} = 10.04, p < 0.005$), with pairwise comparison showing an increase of this parameter in the standard pellet condition ($p < 0.005$), and a main effect of session ($F_{3,96} = 27.30, p < 0.001$). There was also a significant interaction between sessions and reinforcer type ($F_{3,96} = 4.47, p < 0.01$). For the parameter a , there was a main effect of sessions ($F_{3,78} = 4.90, p < 0.005$). With the parameter δ , there was a main effect of sessions ($F_{3,96} = 8.80, p < 0.005$).

4. Discussion

The present findings add to the limited knowledge of the behavioural effects of the high-fat diet model of obesity induced diabetes and of the STZ-induced model. Similar effects with two types of appetitive reinforcer were found across three experiments and across two distinct experimental mouse models of T2DM. In the T-maze study (Experiment 1), there was an effect of food type with a preference for corn oil for both groups of mice, measured by frequency of arm choice associated with either food type. There was also a difference between times taken to consume the food, with corn oil being eaten more quickly than the syrup. Thus, high-fat diet fed mice preferred food that is high in fat content over sugar. However, this pattern of preference was also evident in mice that were maintained on a standard (low-fat) diet. These findings are not inconsistent with results from human studies where people with heavier body weights (Drewnowski et al., 1985; Elfhag & Erlanson-Albertsson, 2006) or with T2DM (Laitinen, Tuorila, & Uusitupa, 1991) exhibit more pronounced preference for fatty foods as opposed to sugary foods. However, it appears that preference for high-fat content foods exists among Swiss TO mice, regardless of whether animals are of normal weight, overweight, or exposed to high-fat or standard diet

Table 2

Mean values of all four parameters of the PR model for vehicle-treated and STZ- treated mice for final five sessions from the post-treatment phase and each of the first 3 extinction sessions. Standard deviation in parenthesis.

Vehicle-treated group			STZ-treated group		
Parameter		Corn oil	Food pellet	Corn oil	Standard pellet
T_0	Post	11.73 (12.43)	11.39 (8.55)	9.78 (7.66)	13.45 (5.88)
	Ext 1	4.99 (4.83)	10.66 (7.95)	10.89 (6.02)	10.61 (8.14)
	Ext 2	8.65 (15.22)	5.14 (9.37)	17.22 (9.51)	10.63 (12.50)
	Ext 3	17.56 (46.71)	5.55 (9.69)	19.12 (17.87)	139.9 (384.5)
k	Post	0.25 (0.17)	0.56 (0.20)	0.23 (0.20)	0.56 (0.17)
	Ext 1	0.19 (0.16)	0.11 (0.28)	0.08 (0.08)	0.15 (0.27)
	Ext 2	0.40 (0.28)	0.55 (0.30)	0.20 (0.15)	0.44 (0.28)
	Ext 3	0.46 (0.19)	0.55 (0.28)	0.39 (0.22)	0.68 (0.24)
a	Post	17.33 (96.49)	33.61 (24.39)	18.65 (19.75)	12.77 (6.21)
	Ext 1	11.42 (10.08)	11.61 (15.04)	7.97 (4.38)	10.53 (7.99)
	Ext 2	8.61 (5.65)	15.92 (27.69)	5.80 (3.89)	4.91 (5.31)
	Ext 3	20.08 (14.42)	10.03 (10.57)	13.38 (9.09)	16.61 (10.52)
δ	Post	3.66 (0.67)	4.91 (1.10)	3.59 (1.14)	3.06 (0.82)
	Ext 1	2.86 (0.60)	3.10 (1.11)	3.15 (0.60)	2.76 (0.56)
	Ext 2	2.77 (0.77)	3.27 (1.22)	2.79 (0.70)	2.05 (0.73)
	Ext 3	5.26 (5.68)	3.74 (1.83)	4.11 (1.35)	7.175.91)

(Yoneda et al. (2007) also found corn oil to be a highly palatable food source for BALB/c mice). This lack of difference occurred despite the marked elevation in blood glucose levels in the high-fat diet group. Mice maintained on a standard laboratory diet remained normal weight, whereas mice maintained on a high-fat diet became obese and displayed hallmarks of T2DM (Winzell & Ahren, 2004). Given the amount of either food provided in each trial was small, the higher preference for corn oil demonstrated by both groups of mice might suggest a taste preference for high-fat food, independent of the post-ingestive effects of either food type. However, recent physiological research indicates another possibility. The gastrointestinal branches of the vagus nerve have long been known to provide feedback leading to cessation of eating, but the vagus nerve has now been implicated in mediating reinforcing effects of specific foods (Han et al., 2018). The gut-brain axis may therefore be responsible for the type of reinforcer preference seen here.

While the degree of preference for taste of corn-oil over syrup did not change with T2DM, this does not necessarily imply that other behavioural processes relating to food intake are not affected by exposure to a high-fat diet. Food consumption may be influenced by multiple factors associated with motivation, such as deprivation and hunger levels, as well as the incentive or reinforcing effects of food (Smith, 2000). Interactions between these factors leads to disruptions to an organism's drive to eat, which can potentially result in overeating and eventual weight gain (Chechlac et al., 2009). Consequently, further studies of motivation in T2DM mouse models were conducted.

The main aim of Experiment 2 was to determine whether changes in motivation, assessed by a PR schedule, occur in the high-fat diet mouse model of T2DM. Despite the physiological indications of the presence of both obesity and T2DM, high-fat feeding did not result in either a consistent increase, or decrease, in the level of motivation to consume the food-pellet or corn-oil reinforcers measured in various ways. Operant responding was maintained at a high level over long periods of time under PR schedules. When stable responding occurred for food-pellet reinforcement, the modal last ratio completed, the break point number was 15. This indicates that the last ratio completed was 95 and a total of 450 operant responses had been made to that point in the PR session. PR schedule behaviour also showed high resistance to extinction. Following training with food-pellet reinforcement, after 11 extinction sessions break point number was typically 8, meaning that on the 11th daily extinction session the last ratio completed was 20 and a total of 69 responses had been made, and following training with corn-oil reinforcement after 5 extinction sessions break point number was again typically 8. All these data are evidence of high levels of motivation to respond for both types of food. The finding that the second series of extinction sessions was more effective in suppressing responding is also typical of what is found is following training on various intermittent schedules with food reinforcement (Leslie, 1996).

Other attempts to reduce motivation had variable effects. The satiation probes, where the normal food deprivation regime was suspended for two days, twice had the expected effect of reducing break point number on the satiation test session which then rapidly recovered. When satiation was attempted by preloading with the reinforcer, this only worked when a very large quantity of corn oil was used. As in Experiment 1, Swiss TO mice seemed averse to consuming large quantities of golden syrup. In previous studies syrup has been used as a reinforcer for mice, although most often it has been diluted (Lewis et al., 2005; Tetri, Basaranoglu, Brunt, Yarian, & Neuschwander-Tetri, 2008). The main finding remains that on many measures of operant responding with two different food reinforcers, there was no difference in the performance of a standard diet fed group and a high-fat fed group of Swiss TO mice that showed physiological signs of diabetes and typically consumed more calories per day. Human obesity, which is a significant precursor of diabetes (Ford, Williamson, & Liu, 1997), is associated with marked preferences for energy dense foods (Berthoud & Zheng, 2012; Drewnowski et al., 1985) and an increased drive to consume foods, even when the individual is satiated (Castellnuovo-Tedesco & Schiebel, 1975; Rosen & Aniskiewicz, 1982). While high-fat mice in this study had abnormally elevated blood glucose concentrations, prolonged feeding on a high-fat diet did not have clear effects on food motivation. This was surprising given that high-fat mouse models continue to show validity in an increasing range of physiological studies of T2DM (e.g., Evans et al., 2014; Lee et al., 2013).

An alternate model was employed next, and one which typically induces a higher degree of hyperglycaemia. Experiment 3 used an STZ-induced mouse model of diabetes and evaluated performance on the same PR schedule using a conventional break point number measure and also a more detailed assessment with Bradshaw and Killeen's (2012) quantitative method of analysis. Mice that received STZ treatment exhibited significantly higher blood glucose levels than mice that received vehicle (Surwit, Kuhn, Cochrane, McCubbin, & Feinglos, 1988). As in previous rodent studies (Pournaghi, Sadrkhanlou, Hasanzadeh, & Foroughi, 2012; Sevak et al., 2007), STZ-treated mice displayed a decrease in body weight over the duration of the study, whereas vehicle treated mice did not. As previously found with rats (Smith & Gannon, 1991), water intake of STZ-treated mice was significantly higher than the vehicle controls. Taken together, these results suggest that the STZ treatment produced a physiological profile associated with the presence of diabetes. The main aim was to evaluate the impact on performance on a PR schedule of food reinforcement, and examine whether motivation to respond was affected by type of food (corn-oil reinforcer or standard pellet reinforcer). The conventional behavioural measure, break point number, did not differ between STZ-induced diabetic mice and their vehicle treated controls, during any phase of the experiment. However, as in Experiment 2, break point number was affected by reinforcer type, and was significantly lower when mice were responding for standard pellets rather than corn-oil, with this difference also being apparent in the pre-treatment phase of the study when all animals were non-diabetic. During extinction sessions, similar results were obtained, with lower break point numbers for mice that had previously been reinforced with standard pellets compared to those mice that had responded for corn-oil. On all these measures, mice were more motivated to respond for corn-oil than standard pellets, regardless of their diabetic or non-diabetic status.

Recent research has questioned if break point on a PR schedule is an accurate measure of motivation, or reinforcer value (Bradshaw & Killeen, 2012). Reasons for this include the fact that the break point is derived from a single data point during each session, ignoring data from the rest of the session (Arnold & Roberts, 1997); there has also been disagreement on defining of the length of time that should pass without a response/completion of a ratio before responding can be said to have stopped (Rickard et al., 2009). Furthermore, the break point is not just sensitive to the motivational effects of an intervention but also to changes in motor ability (Aberman, Ward, & Salamone, 1998; Covarrubias & Aparicio, 2008). For these reasons, additional analysis of PR performance

(Bradshaw & Killeen, 2012) was performed. This differentiated between motor and motivational processes and provided quantitative measures that offer advantages over conventional analysis (Bradshaw & Killeen, 2012; Olarte-Sanchez et al., 2012; Valencia-Torres et al., 2014; 2017) through four parameters that may be sensitive to different independent variables (Valencia-Torres et al., 2014). The α parameter is sensitive to changes in quantity and quality of ingestible reinforcers (Olarte-Sanchez et al., 2012; Rickard et al., 2009). In the present study, α was decreased in animals that were reinforced using standard pellets compared to those that received reinforcement with corn-oil. This is consistent with the view that corn-oil has higher reinforcing value for mice. Surprisingly, the inducement of diabetes through STZ treatment did not affect α , with both treatment groups exhibiting similar performance under the PR schedule. This suggests that STZ-induced T2DM is not associated with a change in the motivation of C57Bl/6 j mice for either reinforcer. This contrasts with Valencia-Torres et al.'s (2014) finding with rats that STZ treatment resulted in decrease in α , thus in motivation, or incentive value of the reinforcer, while motor capacity (δ) was not affected.

The δ parameter relates to the minimum time taken to make a response and is expected to change with variables that influence motor capacity. In the present study, for animals responding for standard pellets the δ parameter was decreased compared to animals reinforced with corn-oil. It is unclear why a highly fatty food would impair operant responding. However, as found previously with rats (Valencia-Torres et al., 2014), STZ-induced diabetes did not affect this parameter to a greater or lesser degree than vehicle treatment. The model distinguishes between pausing between responses, and post-reinforcement pauses, identified by the parameter T_0 . This did not vary systematically with STZ treatment or by reinforcer type. Treatment with STZ did not affect the parameter k either. Experiment 3, like Experiment 2, included a series of extinction sessions. Diabetes is associated with cognitive deterioration (Kodl & Seaquist, 2008), the development of Alzheimer's disease (Cukierman, Gerstein, & Williamson, 2005), and memory deficits (Biessels & Gispen, 2005; Messier, 2005). The extinction process reflects the context-dependent re-learning capacity of an organism (Berman & Dudai, 2001), and thus involves adaptive learning and memory capacity that may be impaired by diabetes. However, similar to Experiment 2, comparisons of performance between the post-treatment phase and extinction sessions demonstrated a similar decline in break point measures for both STZ and vehicle treatment groups, suggesting that STZ-induced diabetes in C57Bl/6 mice does not result in a higher or lower level of resistance to extinction. As in earlier stages of the experiment, mice reinforced with standard pellets reached lower break point numbers than mice previously reinforced with corn-oil. In relation to the quantitative model, the T_0 parameter was not systematically affected by treatment with STZ or by reinforcer type across extinction sessions. In the case of k , STZ treatment also had no effect on this parameter. For both α and δ , parameters associated with motivational and motor factors respectively, a similar pattern of effect was apparent: neither was systematically altered by STZ treatment or reinforcer type during extinction sessions.

Overall, effects of STZ treatment on model parameters were not observed. This may be because of a limited goodness-of-fit of data to the model. In the present study, the number of sessions was restricted (10 pre-treatment, 10 post-treatment, and 4 extinction sessions) due to concerns about the general health of the STZ treated animals, and r^2 ranged from between 0.38 to 0.65. A previous study of effect of STZ-induced diabetes on PR schedule performance used a much larger number of overall sessions (90 pre-treatment sessions and 30 post-treatment sessions) than the current study, which may have increased the likelihood of group differences, particularly as some results only became apparent during sessions 20–30 in the post-treatment phase (Valencia-Torres et al., 2014). However, the mice in the present study responded vigorously in PR sessions: the highest ratio reached was 23, requiring 492 responses, with a cumulative number of 2600 presses in a 1 h session, while rats used in the Valencia-Torres et al. study typically reached the 15th ratio, requiring 95 responses, and a cumulative total to that point of 450 responses. Furthermore, at the pre-treatment stage α was higher in the corn-oil condition, consistent with our other findings and analyses. Overall, these results are consistent with those of Experiments 1 and 2 in showing preference (Experiment 1) or higher reinforcing value (Experiment 2) of corn-oil over food pellets, but no differential effect of a treatment expected to induce T2DM on behaviour during conditioning or extinction. As with high-fat diet, STZ treatment continues to be used as a valid mouse model of T2DM in non-behavioural studies (e.g., Hu, Huang, Lin, & Lin, 2020). It is possible that in this and the previous experiments using the high-fat model, the degree of diabetes induced was below a level that would show effects on the motivation of behaviour. However, this is unlikely as our procedures were similar to those used in many physiological experiments that have established T2DM in mice.

In conclusion, these innovative experiments on behavioural evaluation of mouse models of T2DM have shown that while the strains of mice investigated developed the physiological signs of T2DM, food motivation did not change relative to that of control mice. Mice with induced T2DM consistently showed a similar preference for high-fat reinforcers over high-sugar reinforcers as did control mice, and there was no evidence for a shift in motivational or reinforcing values of these foods during operant conditioning, disruption with satiation, or operant extinction. It was hypothesised in the Introduction that T2DM mice might eat more and gain weight relative to control because insulin insensitivity makes high-fat food more attractive or, alternatively, because cellular glucoprivation makes high carbohydrate foods more valued. However, neither of these hypotheses has been supported because none of a wide range of tests showed either type of reinforcer to be more effective with T2DM mice. It may be that T2DM mice would be shown to be more susceptible than others to reinforcers that combine high fat and high sugar content: it was noted earlier that human obesity is associated with marked preferences for such energy dense foods (Berthoud & Zheng, 2012; Drewnowski et al., 1985).

Author statement

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Formal analysis: Hitchen and Leslie.

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